

I. AMENDMENTS

A. In the Specification

Please delete the previously filed Sequence Listing and insert therefor the substitute Sequence Listing filed herewith.

Please replace paragraph 39 with the following amended paragraph:

[0039] Figure 3 provides the nucleotide sequence and the location of restriction endonuclease recognition sequences of the Multiple Cloning Site of pUni/V5-His version A (SEQ ID NO: 18), and a plasmid map of this 2.3 kb vector. EcoRI cloning site is located at nucleotide 471, and SacI cloning site is located at nucleotide 528. The amino acid sequence of the V5 epitope and 6X His tag (SEQ ID NO:19) also is shown.

Please replace paragraph 40 with the following amended paragraph:

[0040] Figure 4 provides the nucleotide sequence and the location of restriction endonuclease recognition sequences of the Multiple Cloning Site of ~~pCR-2.1~~ the pCR[®] 2.1 vector (SEQ ID NO:17), and a plasmid map of this vector. HindIII cloning site is located at nucleotide 234, SpeI is at nucleotide 258 and EcoRI is at nucleotide 283 and nucleotide 299 (nucleotide positions as in SEQ ID NO:17). The vector is 3906 nucleotides. LacZ alpha fragment: bases 1-587; M13 reverse priming site: bases 205-221; Multiple cloning site: bases 234-355; T7 promoter/priming site: bases 362-381; M13 forward (-20) priming site: bases 389-404; M13 Forward (-40) priming site: bases 408-424; fl origin: bases 546-960; kanamycin resistance ORF: bases 1294-2088; ampicillin resistance ORF: bases 2106-2966; ColE1 origin: bases 3111-3784. The illustrated vector represents the pCR[®]2.1 vector with a PCR product inserted by TA Cloning[®]. Note that the inserted PCR product is flanked on each side by EcoRI sites. The arrow indicates the start of transcription for the T7 RNA polymerase.

Please replace paragraph 43 with the following amended paragraph:

[0043] Figure 7 illustrates the addition of adapter oligonucleotides to the digested vector in the presence of DNA ligase. The reaction yields the exhibited linearized, adapted vector. Adapter sequences are underlined for demarcation. The four adaptor oligonucleotides have the following sequences:

TOPO D1: 5'-AATTGATCCCTTCACCGACATAGTACAG-3' (SEQ ID NO:5)

TOPO D2: 3'-CTAGGGAAGTGG-5' (SEQ ID NO:6)

TOPO [[D3]] D4: 3'-GACATGATACAGTTCCCGC-5' (SEQ ID NO:8)

TOPO [[D4]] D5: 5'-AAGGGCGAGCT-3' (SEQ ID NO:7)

T4 ligation reaction will yield the indicated linearized cloning vector, adapter sequences are underlined for demarcation. Sequences of the adapters with portions of vector sequences are shown (SEQ ID NOS:20 to 23).

Please replace paragraph 46 with the following amended paragraph:

[0046] Figure 10 illustrates that addition of an annealing oligonucleotide to the linearized, adapted vector in the absence of DNA ligase yields the exhibited linearized, adapted and annealed vector. Note that the annealing oligonucleotide is not bound to the vector by a phosphate bond, thus, allowing dissociation following topoisomerase mediated cleavage. Adapter oligonucleotides are demarcated by a single underline, while annealing oligonucleotides are demarcated by a double underline. There are no ~~phosphodiester~~ phosphodiester linkages between either of the TOPO D3s and their adjacent oligonucleotides TOPO D2 and TOPO D5. The annealing oligonucleotide has the following sequence and is complementary to both TOPO D1's and TOPO D4's single stranded overhang: TOPO D3 3'-CTGTATCATGTCAAC-5' (SEQ ID NO:10). Sequences of the adapters with portions of vector sequences are shown (SEQ ID NOS:20, 22 and 24).

Please replace paragraph 51 with the following amended paragraph:

[0051] Figure 13B shows the adapted version of ~~pCR-2.1~~ the pCR[®] 2.1 vector following incubation with the adapter oligos in the presence of T4 ligase; the adapters with portions of vector sequences are shown (SEQ ID NOS:25 to 28).

Please replace paragraph 52 with the following amended paragraph:

[0052] Figure 14 illustrates the addition of annealing oligonucleotides to the adapted pCR2.1[®] vector, followed by the binding of topoisomerase I and the topoisomerase mediated cleavage of the double stranded vector. The resulting vector is linear and charged with topoisomerase I on both ends. Also, one end of the vector has the custom 4 bp single stranded sequence, while the other end is blunt. In the initial reaction illustrated, topoisomerase binds and cleaves the double stranded DNA at the 5' end of the covalent binding site located near the ends of ~~pCR-2.1~~ the pCR[®] 2.1 vector, which contain the bound adapter and annealing oligonucleotides. This step is performed in the presence of T4 polynucleotide kinase. The annealing oligonucleotides have the following sequences:

TOPO 3: 3'-TAAGGCTATCACAAC-5' (SEQ ID NO: 15); and

TOPO 17: 3'-GCTATCAC-5'

There are no ~~phosphodiester~~ phosphodiester bonds formed between TOPO 3 and TOPO 2, or between TOPO 17 and TOPO 16. The annealing oligonucleotides are double underlined for demarcation. The adapters and portions of vector sequences are shown (SEQ ID NOS 25 to 34).